

## Identification of $\alpha$ - $\beta$ unsaturated aldehydes as sources of toxicity to activated sludge biomass in polyester manufacturing wastewater

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### ABSTRACT

Wastewater generated in industrial production processes are often contaminated by hazardous chemicals. Characterization by means of toxicity-directed analysis is useful for identifying which fractions of a waste stream possess the most toxicity. We applied this approach to evaluate toxic components of a polyester manufacturing wastewater. Using the reduction in oxygen uptake rate of activated sludge as an indicator of toxicity, it was determined that increasing the pH from 3 to 11 followed by air stripping significantly reduced the toxicity of the wastewater. Comparative headspace GC/MS analysis of wastewater at different pHs selected a group of Volatile Organic Compounds (VOCs) associated with the observed effect of air stripping at pH 11. Ten of these compounds were identified as  $\alpha$ , $\beta$  unsaturated aldehydes (acrolein (2-propenal) congeners); these compounds are known to be toxic as well as mutagenic. Confirmation that these compounds were a cause of toxicity was achieved by demonstrating that removal of these compounds by air stripping significantly reduced the wastewater mutagenic potency in a *Salmonella* mutagenicity assay. Formation of these volatile compounds by base catalyzed aldol condensation at pH 11 may account for the effectiveness of air stripping in reducing toxicity. To date there is no record in the literature about the toxicity and presence of acrolein congeners in polyester manufacturing wastewater.

**Key words** | acrolein congeners, aldol condensation, 2-butenal, crotonaldehyde, mutagenicity, polyester manufacturing wastewater, 2-propenal, toxicity, toxicity-directed analysis,  $\alpha$ , $\beta$  unsaturated aldehydes

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### INTRODUCTION

Sustainable and cleaner industrial production has become a social priority as reflected by increasingly stringent government regulations. The treatment of wastewater generated in industrial production processes is of particular interest because they are often contaminated by hazardous chemicals and represent a large portion of industrial pollution.

In the past characterization of industrial wastewater with the intent of limiting their environmental impact was

based primarily on physical and chemical factors such as suspended solids, color, Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). In addition, toxic compounds of special concern were monitored through the use of chemical-specific analyses that emphasized priority pollutants. However, this type of targeted analysis may fail to recognize the most harmful toxic wastewater constituents (Reemtsma 2001). It also does not assess the effects of interactions between contaminants.

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Toxicity assessment of whole effluents using biotests is an alternative to chemical target analysis and interactive toxicity between components is addressable with this approach (Eilersen *et al.* 2004). However, biotesting alone is not a sufficient basis for risk reduction measures such as emission control and does not provide information on the compounds causing the measured effects (Chapman 2000; Brack 2003).

In the early 1980's, researchers came up with a new approach called effect-, bioassay- or toxicity-directed analysis, which combines chemical analysis with physico-chemical fractionation and biotesting (Brack 2003). Toxicity-directed wastewater analysis allows one to focus analytical efforts on the most harmful constituents of an effluent (Reemtsma 2001). This approach is particularly useful in situations when little data is available on the components and toxicity of the effluent in question, as is the case for polyester manufacturing wastewater.

Polyester manufacturing wastewater consists of water condensed in step-growth polymerization reactions primarily between diols and dicarboxylic acids. Since it is formed in contact with reactants and products, this water is heavily contaminated. Characteristics of this kind of wastewater reported in the literature are based on physico-chemical properties such as low pH (2.3 to 3.9) and high COD (22,900 to 250,000 mg/L) (Meriç *et al.* 1999; Caffaro-Filho *et al.* 2009) and targeted chemical analysis for specific compounds such as 1,4-dioxane (about 600 mg/L) (So *et al.* 2009).

In the case of treatability studies of industrial wastewater, reduction of toxicity by wastewater-treatment processes is rarely monitored (Köhler *et al.* 2006). Such is the case of polyester manufacturing wastewater.

The main objective of this work was to apply toxicity-directed analysis to a polyester manufacturing wastewater to aid in the evaluation of practical methods to reduce its toxicity toward activated sludge. Using the reduction in oxygen uptake rate of activated sludge as an indicator of toxicity, it was determined that increasing the pH from 3 to 11 followed by air stripping reduced the toxicity of the wastewater by 62%. The results of this toxicity reduction evaluation are reported in another article (Caffaro-Filho *et al.* 2009). The toxic compounds removed by air stripping at pH 11 corresponded to the majority of the components

responsible for the acute toxic effect on the unacclimated activated sludge. The most consistent hypothesis for the identity of these toxic compounds is that they are VOCs poorly volatilized under acidic conditions and highly volatilized under basic conditions. Other possibilities had been previously eliminated, including toxicity due to ammonia and oxidizable compounds (Caffaro-Filho *et al.* 2009). To test this hypothesis and identify the responsible toxic compounds, GC/MS analyses of wastewater headspace were conducted at pH 3 and 11.

## MATERIALS AND METHODS

### Gas chromatography/mass spectrometry analysis (GC/MS)

The static headspace gas chromatographic technique (Franchini *et al.* 2001) was employed for analysis of Volatile Organic Compounds (VOCs) in polyester resin manufacturing wastewater. Gas chromatography/mass spectrometry (GC/MS) was used for analysis of wastewater VOCs released at pH 3 (original wastewater pH); at pH 11; and after 9 hours of air stripping at pH 11 (air was pumped through a porous rock at 3 L min<sup>-1</sup>). Aliquots (10 mL) of each wastewater were transferred to extraction vials and sealed with silicon/PTFE septa. VOCs were extracted by solid phase micro-extraction (SPME) in the vial headspace and analyzed by GC/MS as previously described (Caffaro-Filho *et al.* 2009). The SPME fiber was removed and inserted in the GC injector. VOCs were separated by gas chromatography on a DB-1 (30 m × 0.25 mm × 0.25  $\mu$ m) nonpolar silica capillary column (J&W Scientific, Folsom, California, USA). Column temperature was held at 30°C for 5 min, followed by a temperature program from 30–80°C at 1.5°C min<sup>-1</sup> and then from 80–200°C at 6°C min<sup>-1</sup>, and then held at 200°C for 5 min. The carrier gas was helium at 1.0 mL min<sup>-1</sup> constant flow rate. Total time of each run was 60 minutes.

### Identification of compounds of interest

#### Selection criteria

Based on the GC/MS chromatograms, compounds with the following characteristics were identified as compounds of

interest: those that were major components of the VOCs detected at pH 11; and were absent or greatly reduced at pH 3; and that were reduced by at least 50% after air stripping at pH 11.

### Mass spectra library search

The acquired mass spectra were deconvoluted using an automated mass spectral deconvolution and identification system (AMDIS, National Institute of Standards and Technology, Gaithersburg, MD, USA). All AMDIS deconvoluted mass spectra were then compared to reference spectra in the National Institute of Standards and Technology mass spectral library (NIST 98) for tentative identification (Grung *et al.* 2007). Compounds that presented no reasonable match in the mass spectral library were excluded from further investigation steps.

### Toxicological literature survey

After selecting the best hits on the NIST mass spectral database, a toxicological literature survey on the tentatively identified compounds was made to assess if these compounds had a relevant toxicity record. Compounds that had no references in the toxicological literature were excluded from further investigation steps.

### Kováts retention indices database survey

Kováts retention indices (KI) were calculated for the remaining suspected compounds. A search for KIs of reference compounds obtained with similar capillary columns and temperature programs, i.e. non-polar columns with a temperature ramp program, was performed using the following three electronic databases: Flavornet (Acree & Heinrich, accessed in 2006); The Pherobase (El-Sayed, accessed in 2006); and NIST Chemistry WebBook (Linstrom & Mallard 2005). Compounds of interest with retention indices closely matching a Kováts index reference compounds were considered identified.

### *Salmonella* mutagenicity assay

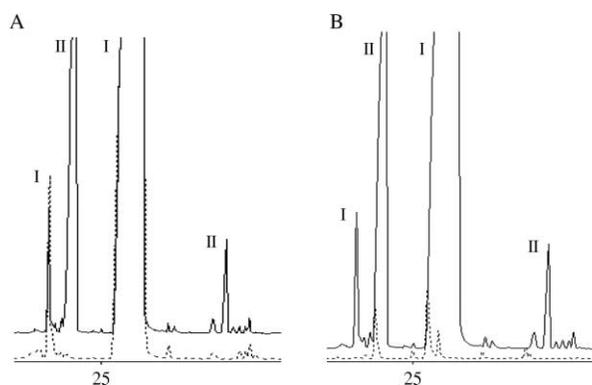
The purpose of this assay was the confirmation of an identified compounds mutagenic activity. The identified

toxic compounds are also known to be mutagenic. It was hypothesized that if they were in fact volatilized by air stripping at pH 11 but not significantly at pH 3, there should be a difference in mutagenicity between these two fractions (mutagenicity of the pH 3 stripped fraction should be higher). Air stripped pH 3 and pH 11 wastewater samples were tested in the *Salmonella typhimurium* microsome assay (Maron & Ames 1983) after membrane filtration to remove microorganisms and concentration *in situ* using the protocol developed by the Coriell Institute for Medical Research (1986). The *Salmonella* strains used were TA100 and TA104 kindly supplied by Dr. B. N. Ames. TA104 is known to be particularly sensitive to genotoxic aldehydes (Levin *et al.* 1982). The experiments were performed with and without rat liver S9 fraction using a pre incubation time of 30 min at 37°C. The plates were incubated for 66 hours. The S9 mix was freshly prepared before each test using lyophilized Aroclor-1254-induced rat liver S9 fraction (Moltox-Molecular Toxicology Inc., Boone, NC, USA). The doses tested were 0.1; 0.2; 0.5; 1.0 and 2.0 mL/plate. Results were statistically analyzed with the SALANAL computer program (designed and kindly provided by L. Myers from Integrated Laboratory Systems, North Carolina, USA) using the Bernstein model (Bernstein *et al.* 1982), and potencies were expressed in revertants (rev)/L. The assay was performed in triplicate for each dose tested.

## RESULTS AND DISCUSSION

### Compounds of interest selection

The chromatograms from GC headspace analysis showed 161 resolved peaks from pH 3 wastewater, 185 peaks from pH 11 wastewater and 97 peaks from pH 11 wastewater after 9 hrs of air stripping. A total of 22 peaks (considered compounds), identified in the pH 11 chromatogram, were selected (as described in methods) to be in the list of compounds of interest and to be identified further. Limited sections of GC chromatograms are shown in Figure 1 as examples of the superimposition of chromatograms from different samples used to identify compounds for addition to the list of compounds of interest.



**Figure 1** | Superimposition of chromatograms. (A): pH 11 chromatogram (solid line) and pH 3 chromatogram (dotted line) showing examples of [I] excluded compounds and [II] compounds of interest; (B): Superimposition of pH 11 chromatogram (solid line) and air stripped pH 11 chromatogram (dotted line) showing examples of [I] excluded compounds and [II] compounds of interest.

## Identification of compounds of interest

### Mass spectra library search

Mass spectra library matches for compounds of interest are shown in Table 1. Compounds are ordered by GC peak area. Nine compounds had no reasonable match from the library used in this study, and were excluded from further investigation steps. Unfortunately, five of them were among those with the greatest peak areas, which in turn was a rough indication of compound concentration in the sample.

### Toxicological literature survey

A toxicological literature survey on compounds 9, 14 and 19 (propylene glycol; bicycle [3.1.0] hexan-3-one; 2-ethyl-4-methyl-1-pentanol) revealed no relevant toxicity record in the context of this research. These compounds were therefore excluded from further investigations.

The remaining 10 compounds belong to the chemical class of  $\alpha,\beta$ -unsaturated aldehydes (acrolein congeners). These compounds are important industrial chemical compounds, are formed by the combustion of organic material and are commonly occurring environmental pollutants (Eder & Deininger 2002). Compounds of this group are known for aquatic toxicity (Katritzky & Tatham 2001; Dimitrov *et al.* 2004), cytotoxicity (Kaneko *et al.* 1987; Niknahad *et al.* 2003) and mutagenicity (Benigni *et al.* 2003). In addition, a survey of the pollutants generated by

the Plastic Resin and Manmade Fibers industry by the USEPA (1997) cites crotonaldehyde (2-butenal synonym) as a contaminant released by fugitive air emissions. Therefore, these compounds were maintained for subsequent investigation.

### Kováts retention indexes database survey

Retention indexes provide valuable additional information on compounds identity (Brack 2003). The Kováts retention indexes (KI) for the remaining tentatively identified compounds, along with KIs and CAS numbers of these compounds in chemical databases, are shown in Table 2. Index search was restricted to those obtained using similar GC conditions, i.e. non-polar columns with a temperature ramp program.

Compounds identified by MS and presenting a high KI index similarity with literature KIs were regarded as identified and were 2-butenal, 2-methyl 2-butenal [M], 2-methyl 2-butenal [R], 2-pentenal, 2-methyl 2-pentenal and 2,4-hexadienal.

### *Salmonella* mutagenicity assay

Results of the mutagenicity assays with wastewater after stripping of VOCs at pH 3 and after stripping of VOCs at pH 11 were statistically analyzed by SALANAL computer program. Both samples exhibited a significant positive dose response and therefore, potencies could be calculated. Results are shown in Table 3. Since the identified compounds were known to be mutagenic to *Salmonella* and are not removed by stripping in acidic conditions, the pH 3 air stripped sample should have been more mutagenic than the pH 11 air stripped sample.

Mutagenicity of both samples was high. However, the pH 11 air stripped sample was significantly less mutagenic (i.e. lack of overlap at the 90% confidence intervals) in three of the four conditions tested (TA100, TA100 + S9 and TA104). Mutagenic potency of the pH 3 stripped sample was  $1.04 \times 10^6$  revertants per liter (rev/L) with strain TA100, while that of the pH 11 stripped sample was  $0.88 \times 10^6$  rev/L. There was a difference of  $1.5 \times 10^5$  rev/L between these two samples, although both values are still in the category of high mutagenic potential according

**Table 1** | Compounds of interest list, showing areas and retention times in headspace GC/MS analyses and mass spectra library matches. Compounds were ordered by area size

Compound	pH 11 headspace chromatogram		pH 3 headspace chromatogram		Stripped chromatogram		Library match
	Area	Retention time	Area	Retention time	Area	Retention time	
1	223,212,048	23,543	ND	ND	6,066,063	22,900	–
2	127,328,731	4,667	ND	ND	ND	ND	2-Methyl 2-butenal [M]*
3	123,207,534	2,635	ND	ND	ND	ND	2-Butenal (crotonaldehyde)
4	48,802,079	43,904	ND	ND	567,822	43,754	–
5	19,636,129	32,660	ND	ND	ND	ND	–
6	13,626,734	40,850	ND	ND	ND	ND	–
7	13,297,402	18,528	ND	ND	ND	ND	–
8	12,638,453	18,094	ND	ND	ND	ND	2,4-Heptadienal
9	10,290,513	7,320	ND	ND	ND	ND	Propylene glycol
10	9,772,061	1,856	474,687	1,870	1,590,666	1,865	2-Methyl 2-propenal
11	7,265,271	4,712	ND	ND	ND	ND	2-Methyl 2-butenal [R]*
12	4,956,139	4,864	ND	ND	ND	ND	2-Pentenal
13	4,889,193	7,766	ND	ND	ND	ND	2-Ethyl 2-butenal
14	4,792,073	10,410	ND	ND	235,200	10,408	Bicyclo [3.1.0] hexan-3-one
15	4,295,606	8,543	ND	ND	ND	ND	2-Methyl 2-pentenal
16	3,707,539	31,844	ND	ND	ND	ND	–
17	3,129,702	10,079	ND	ND	ND	ND	–
18	2,838,783	8,922	ND	ND	ND	ND	2-Ethenyl 2-butenal
19	2,789,209	22,643	ND	ND	ND	ND	2-Ethyl-4-methyl-1-pentanol
20	1,426,953	13,416	ND	ND	ND	ND	2,4-Hexadienal
21	1,124,999	41,262	ND	ND	ND	ND	–
22	1,008,896	29,485	ND	ND	ND	ND	–

\*Isomers.

ND, Not detected in chromatogram; –, No reasonable match in mass spectra library.

to Houk (1992). These results are consistent with the identification of the toxic VOCs as belonging to the class of  $\alpha,\beta$ -unsaturated aldehydes.

### Final considerations

Mutagenicity reduction by removal of the identified toxic compounds is consistent with their identification as a group of  $\alpha,\beta$ -unsaturated aldehydes. In addition, the mutagenicity values obtained with strain TA104 were more than twice those obtained with TA100, which also supports this identification since TA104 is known to be particularly sensitive to genotoxic aldehydes (Levin *et al.* 1982). The  $\alpha,\beta$ -unsaturated aldehydes are direct-acting mutagenic agents, and S9 addition reduces their mutagenic activity

(Eder & Deininger 2001), which was observed in the results obtained and thus is another corroboration of their correct identification. The relatively high mutagenicity of the pH 11 air stripped sample suggests that the remaining mutagenicity is likely to be caused by related compounds that were not adequately volatilized under the conditions employed.

Crotonaldehyde (2-butenal) and the closely related 2-methyl 2-butenal showed peaks with areas greater than all the other identified  $\alpha,\beta$ -unsaturated aldehydes (Table 1) and thus are expected to be present in greater concentrations than the other  $\alpha,\beta$ -unsaturated aldehydes in the effluent. Many researchers have studied crotonaldehyde and specific information on this compound is available in the toxicological literature. Therefore, it was chosen as a representative of the overall toxicity caused by the identified

**Table 2** | Calculated and databases Kováts Indexes (KI) for compounds which had reasonable mass spectra library matches

Compound	Name	CAS	KI calculated	KI database	Status
2	2-Methyl 2-butenal [M]	497-03-0	727	715 <sup>*</sup> ; 724 <sup>*</sup>	Identified
3	2-Butenal (crotonaldehyde)	4170-30-3	631	622 <sup>*</sup> ; 629 <sup>*</sup> ; 648 <sup>*</sup>	Identified
8	2,4-Heptadienal	4313-03-5	942	979 <sup>†</sup> ; 980 <sup>†</sup> ; 983 <sup>‡</sup>	Tentatively identified
10	2-Methyl 2-propenal	78-85-3	< 600 <sup>§</sup>	531 <sup>*</sup> ; 553 <sup>*</sup>	Tentatively identified
11	2-Methyl 2-butenal [R]	1115-11-3	728	722 <sup>†</sup> ; 736 <sup>‡</sup>	Identified
12	2-Pentenal	1576-87-0	733	737 <sup>‡</sup>	Identified
13	2-Ethyl 2-butenal	19780-25-7	798	NF	Tentatively identified
15	2-Methyl 2-pentenal	623-36-9	813	804 <sup>*</sup> ; 808 <sup>*</sup> ; 811 <sup>*</sup>	Identified
18	2-Ethenyl 2-butenal	20521-42-0	820	NF	Tentatively identified
20	2,4-Hexadienal	142-83-6	884	878 <sup>*</sup> ; 881 <sup>*</sup> ; 883 <sup>*</sup>	Identified

<sup>\*</sup>NIST Chemistry WebBook.

<sup>†</sup>The Pherobase.

<sup>‡</sup>Flavornet (interpolated KIs).

<sup>§</sup>Not calculated because compound retention time was less than first standard alkane (C<sub>6</sub>).

NF—Not found.

$\alpha,\beta$ -unsaturated aldehydes. The decrease in mutagenicity by the removal of compounds of interest in the case of TA100 was  $1.57 \times 10^5$  rev/L. Crotonaldehyde (C<sub>4</sub>H<sub>6</sub>O) has a mutagenic potency of  $1.95 \times 10^5$  rev/ $\mu$ mol in *Salmonella* TA100, according to Benigni *et al.* (2003). Therefore, the observed decrease in mutagenicity could have been caused by the removal of an initial concentration of 5.64 mg of crotonaldehyde per liter.

In another study, the experimental acute toxicity (14 day LC<sub>50</sub>) of crotonaldehyde to the guppy (*Poecilia reticulata*) was determined to be 7.94  $\mu$ mol/L, which is equivalent to 556  $\mu$ g/L (Deneer *et al.* 1988). It is possible that a concentration ten times higher (5.64 mg/L), estimated to be the concentration of crotonaldehyde equivalent removed by air stripping at pH 11, could cause the observed acute toxicity of wastewater to unacclimated activated sludge.

In a comprehensive review on the genotoxicity of industrial wastes and effluents, Houk (1992) classified *Salmonella* mutagenic potencies of whole effluents from low (< 10<sup>3</sup> rev/L) to extreme (> 10<sup>7</sup> rev/L), based mainly on results with TA100 strain. All but four of the effluents reviewed fell below the 10<sup>6</sup> rev/L range. In the category of plastics, resins and rubber manufacturers, extreme genotoxicity was related with solid wastes such as plastic tar, which required metabolic activation (S9 addition) to generate metabolites that were more potent than the parent constituents. In the same review, the surveyed wastewater from the production of plastics and resins were variable in their response, and those that were genotoxic were not particularly potent relative to other industrial wastewater. In the case of the wastewater evaluated in this study, the mutagenic potency found (10<sup>6</sup> rev/L) is higher

**Table 3** | Mutagenic potency and 90% confidence intervals calculated by the Bernstein model in SALANAL computer program

Test strains	pH 3 Stripped sample		pH 11 Stripped sample	
	Revertants per liter	90% confidence interval	Revertants per liter	90% confidence interval
TA100	$1.04 \times 10^6$	$9.61 \times 10^5 - 1.12 \times 10^6$	$8.84 \times 10^5$	$8.18 \times 10^5 - 9.50 \times 10^5$
TA100 + S9	$5.24 \times 10^5$	$4.80 \times 10^5 - 5.68 \times 10^5$	$4.07 \times 10^5$	$3.53 \times 10^5 - 4.61 \times 10^5$
TA104	$2.67 \times 10^6$	$2.56 \times 10^6 - 2.78 \times 10^6$	$2.36 \times 10^6$	$2.19 \times 10^6 - 2.53 \times 10^6$
TA104 + S9	$1.42 \times 10^6$	$1.20 \times 10^6 - 1.64 \times 10^6$	$1.27 \times 10^6$	$1.08 \times 10^6 - 1.45 \times 10^6$

than the majority of effluents reviewed by Houk (1992). In addition, the genotoxicity was not dependent on S9 activation.

$\alpha,\beta$ -Unsaturated aldehydes are not used as raw material for polyester resin production. However, they are by-products of the reaction in some cases, which in the case studied occurs at high temperature (200°C). These compounds can also be formed by incomplete oxidation of diols such as 1,4-butanediol. However, to the best of our knowledge, there is no record in the literature about their presence in polyester manufacturing wastewater.

It was beyond the scope of the research presented here to determine why an increase in pH resulted in an increase in the amount of  $\alpha,\beta$ -unsaturated aldehydes observed. However, one possible explanation is that these compounds were produced as a result of base catalyzed aldol condensation reactions between toxic aldehydes in the wastewater. Aldehydes, notably acetaldehyde and formaldehyde, are known byproducts of polyester synthesis (Scheirs & Long 2003). Aldol condensations are well known and involve the reaction of two aldehydes with formation of  $\alpha,\beta$ -unsaturated aldehydes with carbon number equal to the combined carbon numbers of the two reacting aldehydes. Most commercial applications use base-catalyzed aldol condensation reactions (Serra-Holm *et al.* 2000). For example acrolein is commonly made by the aldol condensation of formaldehyde with acetaldehyde and 2-butenal (crotonaldehyde) by the aldol condensation of acetaldehyde (Azzouz *et al.* 2003). Moreover, it is known that the formation of crotonaldehyde via aldol condensation in starch hydrolyzates does not occur significantly until pH 7.5 and above (Byrne 1989). In addition, the observed diene  $\alpha,\beta$ -unsaturated aldehydes such as 2,4-hexadienal can be accounted for by the aldol condensation of  $\alpha,\beta$ -unsaturated aldehydes (in the case of 2,4-hexadienal this would be crotonaldehyde) and another aldehyde (in this case acetaldehyde) (Serra-Holm *et al.* 2000). If the  $\alpha,\beta$ -unsaturated aldehydes observed at pH 11 were primarily produced by base catalyzed aldol condensation it would help explain their abundance at pH 11 and not at pH3. Moreover, the  $\alpha,\beta$ -unsaturated aldehydes detected are considerably less soluble in water than formaldehyde and acetaldehyde and therefore should be stripped out more readily, which could

explain the observed reduction in toxicity and mutagenicity as result of air stripping at pH 11.

## CONCLUSIONS

The polyester manufacturing wastewater evaluated in this study was toxic toward unacclimated activated sludge and highly genotoxic in a *Salmonella* mutagenicity assay. Toxicity-directed analysis of a polyester manufacturing wastewater (reported in Caffaro-Filho *et al.* 2009) showed that VOCs removed by air stripping at pH 11 reduced toxicity toward unacclimated activated sludge biomass by 62% in comparison to a 13% reduction at pH 3. Mutagenicity was also reduced in the *Salmonella* mutagenicity assay. Comparative GC/MS analysis of wastewater headspace VOCs at pH 3 and 11 indicated 22 compounds as potential causative agents of toxicity. Ten of them were identified as belonging to the group of  $\alpha,\beta$ -unsaturated aldehydes. These compounds can be formed during the polymerization process; however, to date there is no record in the literature about their effect on toxicity or even their presence in polyester manufacturing wastewater. Alternatively, these compounds may have been formed at pH 11 by base catalyzed aldol condensation reactions of acetaldehyde, formaldehyde and other aldehyde manufacturing byproducts in the original wastewater, which are also toxic mutagenic compounds. The  $\alpha,\beta$ -unsaturated aldehydes detected are considerably less soluble in water than formaldehyde and acetaldehyde and therefore should be stripped out more readily, which could explain the observed reduction in toxicity and mutagenicity after air stripping at pH 11. Further work is needed to evaluate this possibility and to determine if this approach could provide the basis for the development of practical treatment strategies for this kind of toxic effluent.

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